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Ginsenoside Reshapes Intestinal Microecology to Alleviate Microgravity Stress

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Abstract

Background

During medium- to long-duration spaceflights, real-time microgravity can increase the health risks of astronauts. In particular, the disruption of intestinal homeostasis is closely related to other health problems, and it is necessary to monitor related treatment strategies. Ginseng is a well-known Chinese herbal medicine often used to maintain health. Ginseng total saponins (GTSs), which are the bioactive components of ginseng, have been reported to regulate immune homeostasis, anti-inflammation, and anti-oxidation. This study focused on the regulation of GTSs in intestinal homeostasis imbalance caused by microgravity.

Methods

A hindlimb suspension (HLS) rat model was established to evaluate the intestinal protective effects of GTSs.

Differentially expressed genes (DEGs) were screened using RNA-Seq. RT-PCR was performed to further focus and verify these results. The gut microbiome composition was examined based on 16S rRNA gene amplicon sequencing, and the short-chain fatty acids produced were further analyzed.

Results

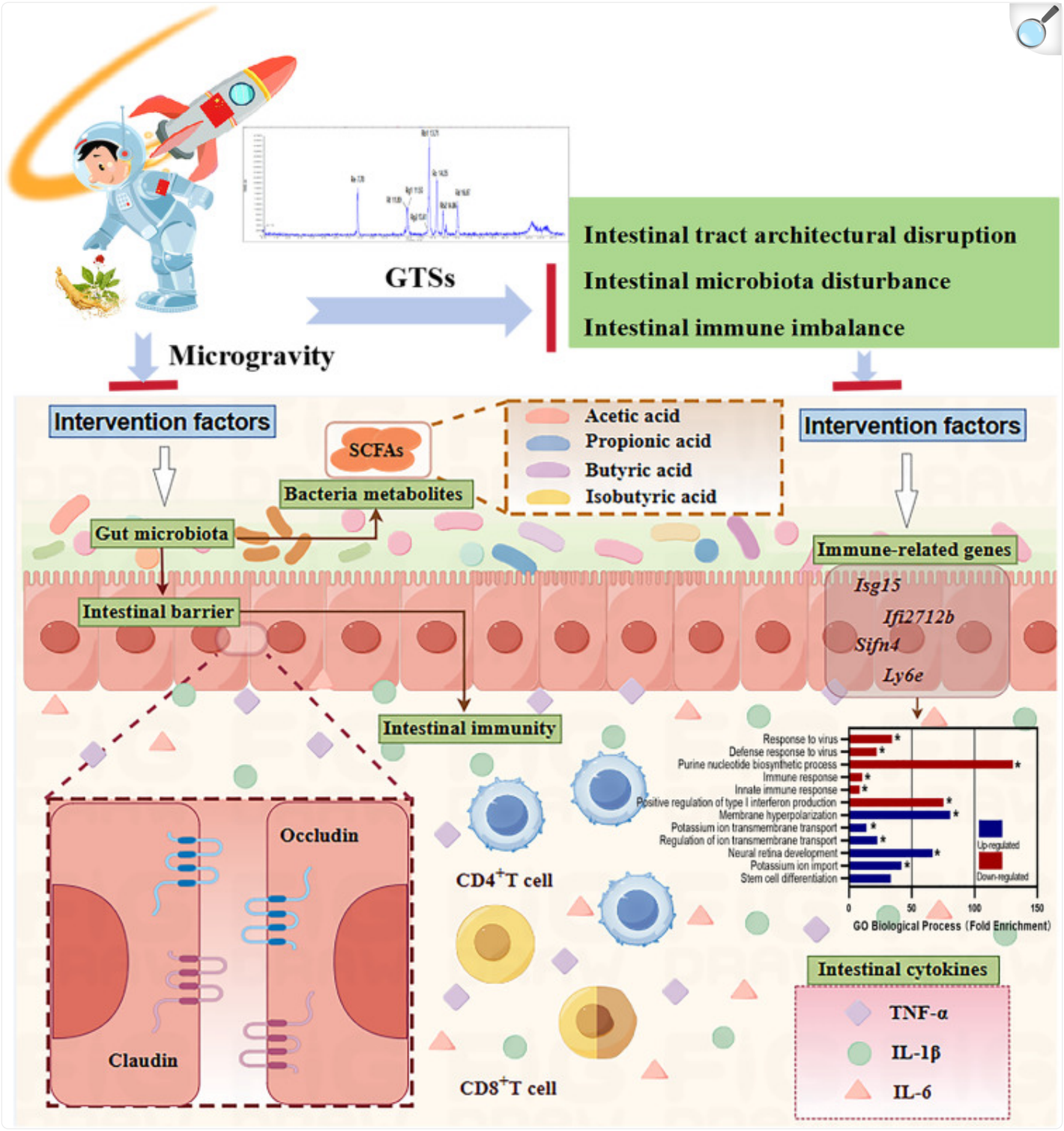
We found that GTSs intervention effectively improved the intestinal injury caused by simulated weightlessness, including reducing the pathological damage, increasing the expression of tight junction proteins and reducing the levels of inflammatory factors. Moreover, GTSs treatment significantly restored the levels of intestinal immunity-related genes and remodeled the gut microbiota. In particular, GTSs significantly increased the abundance of short-chain fatty acid metabolism-related bacteria, thereby increasing the level of propionic acid, butyric acid, isobutyric acid.

Conclusion

Our results revealed that GTSs improved intestinal microecological disorders and impaired immune function caused by the weightlessness simulation. The underlying mechanism may be related to the “intestinal immune -microbiota-metabolic” pathway. These findings provide a theoretical basis for the precise design and development of GTSs for space-health products.

Keywords: ginseng total saponins, gastrointestinal microbiome, immunity, short-chain fatty acids, weightlessness simulation

Graphical Abstract



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Introduction

Long-duration spaceflight missions face challenges due to a variety of factors, including high vacuum, intense radiation, and low magnitude of gravity. Microgravity exposure is the main difficulty that needs to be overcome in real time, which causes astronauts to be in sub-health for a long time, affecting flight tasks and physical fitness.[1] Several studies have shown that microgravity is closely related to health problems such as immune system disorders, cardiovascular dysfunction, and skeletal muscle loss. Intestinal microecology plays an important role in mediating the decline in these functions.[2] Under weightlessness conditions, the intestine directly suffers from structural damage caused by various stress factors, including disruption of intestinal barrier integrity and gut microbiota imbalance.[3,4] Moreover, the gut also regulates immune function and affects signal transduction between the host and intestinal microorganisms by producing intestinal microbial metabolites such as SCFAs.[5,6] Evidence from animal experiments has reported serious intestinal barrier damage under weightlessness simulation, including typical intestinal villi injury, downregulation of tight junction protein expression, cell apoptosis, and increased intestinal permeability.[3] Similarly, shortened intestinal villi, shallower crypt depth, and reduced mucin production in intestinal epithelial cells were observed in spaceflight rats.[7,8] Recent data also show that the fluctuation of intestinal flora caused by weightlessness will lead to the imbalance of the immune system and increase the risk of opportunistic infection. A study revealed that weightlessness caused change in the ratio of Bacteroides to Firmicutes, which may lead to the production of inflammatory cytokines by activating pattern recognition receptors[8]. In addition, microbiota metabolites also participate in the pathological changes of the digestive system related to spaceflight stress and play a role in the body signal transduction. Evidence shows that long-duration space flight significantly changes the gastrointestinal microbiota and related microbial metabolites of astronauts.[9] The exploration based on animal experiments has also identified changes in intestinal metabolome such as amino acids and short-chain fatty acids, which are related to immune dysfunction and intestinal flora disorders.[5,6] Thus, it is necessary to monitor intestinal microecological disorders caused by weightlessness and to discover related protective strategies to ensure the health of astronauts.

In recent years, natural ingredients derived from plants or food, with the advantages of mild antibacterial activity, reparative action, and low resistance to human drugs, have shown broad application prospects in regulating the intestinal microecological balance.[10] As a perennial plant with homology of medicine and food, ginseng has been used for thousands of years in many countries to maintain health. Ginseng total saponins (GTSs), a bioactive compound in ginseng, is responsible for most of the immunological and pharmacological activities of ginseng.[11] According to previous records, GTSs have health benefits in regulating intestinal homeostasis, anti-inflammatory, antioxidant, and anti-central nervous system diseases, and in maintaining immunity.[12] In particular, the effects of GTSs on intestinal immune regulation and inhibition of inflammation have been confirmed.[13] GTSs are also beneficial for increasing the diversity of the gut microbiota, enhancing the abundance of beneficial bacteria (*Bifidobacterium, Bacteroides*) and reducing the production of pathogenic bacteria (*iron-removing bacteria, Helicobacter pylori*) to enhance host health. In addition, the monomeric constituents of ginseng (including ginsenosides Rb1, Rg1, Rk3, and Re) can remodel the composition of the intestinal flora or modulate flora metabolites, thereby causing different effects on the intestinal barrier function, immune function, and host homeostasis.[14–17]

Because of its beneficial effects, CHM Taikong Yangxin prescription, which contains ginseng, has been used to keep Chinese astronauts healthy during their flights. In a previous study, we explored the potential of GTSs to maintain the neurological subhealth of astronauts.[18] However, the regulatory effect of ginseng on intestinal homeostasis under weightlessness conditions remains unclear, and the underlying mechanisms related to gut-brain interactions remain to be explored. Therefore, we used the hindlimb suspension (HLS) model to evaluate the potential and possible mechanisms of GTSs in maintaining intestinal homeostasis, which will help to expand the health effects of ginseng and provide novel understanding and treatment options for health problems, starting with intestinal homeostasis during space travel.

Materials and Methods

Reagents and Chemicals

The GTSs, prepared according to previously published methods,[19] were provided by the Institute of Medicinal Plants, Chinese Academy of Medical Sciences. Ginsenosides Rb1 (Cat no. RM0502), Rb2 (Cat no. RM0503), Rg1 (Cat no. RM0509), Rg2 (Cat no. RM0510), Rc (Cat no. RM0505), Rd (Cat no. RM0506), Re (Cat no. RM0507), and Rf (Cat no. RM0508) were obtained from RefMedic (Sichuan, China). TNF-α (Cat no. 1317202) and IL-6 (Cat no. 1310602) ELISA kits were bought from Dakewei Biotechnology Co., Ltd. (Shenzhen, China).

Anti-occludin (Cat no. 27260-1-AP) and anti-claudin-1 (Cat no. 28674-1-AP) antibodies, HRP-conjugated Goat Anti-Rabbit IgG (H+L) (Cat no. SA00001-2), GAPDH (Cat no. 10494-1-AP) were obtained from proteintech (Wuhan, China).

Acetic acids (Cat no. A298827), propionic acids (Cat no. P110446), butyric acids (Cat no. B110440), and isobutyric acids (Cat no. B1103522) were obtained from Aladdin Scientific (Shanghai, China).

Animals

Specific pathogen-free male Wistar rats (200±20 g) were purchased from Beijing Charles River Experimental Animal Company [SCXK (Beijing, China) 2016–0006]. The animals were kept at a 12 h light/dark cycle environment with a humidity of 45–50% to acclimate for 5 days. All experiments were conducted according to the Principles of Laboratory Animal Care (NIH publication No.86–23, revised in 1996) and approved by the Institute of Medicinal Plants, Chinese Academy of Medical Sciences Animal Care and Use Committee [SYXK (Beijing, China) 2017–0020].

HLS Model

Rats were randomly divided into three groups (n=6): control (CON), hindlimb suspension (HLS), and the HLS treated with GTSs. The HLS model was established based on previous reports.[20] Briefly, the hindlimbs were attached to a separate device with tape and chain hooks to form a 30-degree head-down tilt for 28 days. The CON and HLS groups were administered the same volume of water or GTSs (200 mg/kg, *ig*) daily. Subsequently, feces were collected for 16s rDNA sequencing on day 25, and serum and intestinal tissues were collected for other analyses on the last day.

Qualitative Analysis of Components in GTSs

Ultra-high-pressure liquid chromatography coupled with an electrospray ionization source and a triple-quadrupole mass spectrometer (UPLC-ESI-QqQ-MS/MS), which contained an AB Sciex LC-MS system QTRAP® 6500, was used for separation and quantification. The samples were eluted using an ACQUITY UPLC BEH Shield RP18 column (2.1 mm×50 mm, 1.7 μm particles). The mobile phase consisted of solvent A (0.1% formic acid in water) and B (acetonitrile) and ran under the following program: 0~5 min, 10%~23% B; 5~15 min, 23%~35% B; 15~20 min, 35~48% B; 20~20.5 min, 48%~90% B; 20.5~21.5 min, 90% B; 21.5~23 min, 90%~10% B; 23~25 min, 10% B. The flow rate was set at 0.3mL/min, and the injection volume was 5μL. The column temperature was maintained at 30 °C, and the samples were stored at 4 °C throughout the analysis. Electrospray ion source (ESI) and MRM were performed in the negative mode using nitrogen as the drying agent. The curtain gas (N2) was 40 psi, the collision gas (N2) was 9 psi, and the spray voltage was 5500 V. The atomization temperature was 550 °C, atomization gas (ion source gas 1, N2) and auxiliary gas (ion source gas 2, N2) were used. N2) were both at 55 psi. The corresponding ion pairs and cone voltages are listed in Table S1 .

Histological Examination

Fresh small intestinal tissue from the same part was rinsed with normal saline and fixed in 10% formaldehyde solution until analysis. H&E and AB-PAS staining were performed to observe the tissue pathology, inflammatory responses, and goblet cell hyperplasia.

Flow Cytometry Analysis

Mesenteric lymph nodes were prepared as single-cell suspensions and subsequently incubated with anti-CD3-FITC, anti-CD4-APC, anti-CD8-PE, and anti-CD45-APC antibodies (Biolegend, San Diego, CA, USA) according to the manufacturer's instructions. The stained cells were analyzed using a CytoFLEX Flow Cytometer (Beckman Coulter, USA).

Real-Time PCR

Gene expression levels were measured by real-time PCR. Briefly, total RNA was extracted from small intestinal tissue and reverse-transcribed into cDNA. The primers used are listed in Table S2 . Data were calculated using the $2^{-\Delta\Delta CT}$ method with GAPDH as the internal reference for normalization.

Transcriptome Analysis of Small Intestinal Tissue

Small intestine sections were collected from the Con and HLS groups and transcriptome sequencing was performed using an Illumina HiSeq sequencer by Beijing Boyun Huakang Gene Technology Co., Ltd. Raw data from transcriptomic experiments were deposited in the NCBI SRA database (BioProject ID: PRJNA944741). The raw reads were filtered, and the clean reads were mapped to the rat reference genome (rn6) using HISAT software. Gene expression was quantified by fragments per kilobase of exon per million mapped values (FPKM), and the PossionDis algorithm was used to identify differentially expressed genes (DEGs; log2(fold change) ≥1 and q value ≤0.001). DEGs were then subjected to GO functional annotation. RT-PCR analysis was performed to further screen the DEGs regulated by the GTSs. Primer sequences used are listed in Table S2 .

Gut Microbiota Structural Profiling

Total DNA was isolated from rat fecal pellets by using cetyltrimethylammonium bromide (CTAB)/sodium dodecyl sulfate (SDS). Briefly, 1% agarose gel was used to monitor DNA concentration and purity. According to the concentration, DNA was diluted to 1 ng/μL by adding sterile water. MiSeq systems based on 16S rRNA genes were used to analyze the gut microbiota. Polymerase chain reaction (PCR) was used to amplify specific V3-V4 variable regions using barcode and high-frequency primers. A agarose gel electrophoresis (2%) and AxyPrepDNA (Axygen Scientific Inc. USA) gel recovery kit was used to detect PCR products and recover PCR fragments. According to the preliminary electrophoresis results, QuantiFluor™-ST blue fluorescence quantitative system (Promega Company) was utilized to detect and quantify the products of PCR amplification. The samples were mixed in equal proportions and subjected to the Ultra™ DNA Library Prep Kit (NEB, USA) to construct the libraries. The library was sequenced on a computer following a quality check performed using an Agilent Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) and a qubit. Raw data from 16S rRNA experiments were deposited in the NCBI SRA database (BioProject ID: PRJNA941119).

The library was sequenced using the Illumina MiSeq (Illumina, Inc., San Diego, CA, USA). Paired-end reads from the original DNA fragments were merged using FLASH software. The sequences were analyzed using UPARSE Version 7.0, with the UPARSE-OTU and UPARSE-out ref algorithms. In-house Perl scripts were used to analyze the diversity values of alpha (within samples) and beta (among samples). An in-house Perl program was used to assign sequences to the same operational taxonomic units (OTUs).

SCFAs Analysis

Quantitative analyses were performed as previously described by our group. The fecal sample (~10 mg) was thawed on ice and 200 μL of 50% methanol was added to prepare the homogenate. After centrifugation (4°C13,000 rpm, 10 min), 8 μL of the supernatant was collected. 50% methanol (72 μL), 1M EDC (10 μL), 1M O-BHA (10 μL), and 500 nM Acetic Acid-1-13C (5 μL; IS) were mixed with the supernatant. Derivatization was performed at 25 °C for 1h. Subsequently, 600 μL dichloromethane was added for extraction and 400 μL of the lower organic phase was collected and dried at 37 °C. Finally, 200 μL of 50% methanol was added to the sample, re-dissolved, and centrifuged, and the resulting supernatant was used to re-dissolve the sample. The supernatant was used for LC-MS analysis (AB Sciex LC-MS System QTRAP® 6500).

Chromatographic separation was achieved using an ACQUITY UPLC HSS T3 column (2.1mm×100mm, 1.8 μm) at 50°C with a typical flow rate (200 nL/min). The mobile phase consisted of 0.1% formic acid in water (solvent A) and isopropanol (solvent B). The linear gradient was set as follows: 85%–85% A (0.0 to 1.0 min), 85%–64% A (1.0 to 8.0 min), 64%–50% A (8.0 to 8.5 min), 50%–50% A (8.5 to 10.5 min), and 50%–85% A (10.5 to 15 min). Mass spectrometry (MS) analysis was performed in the positive ion mode with an electrospray ion source (ESI) and MRM mode using nitrogen as the drying agent. The curtain gas (N2) was 35 psi, collision gas (N2) was 9 psi, and the spray voltage was 4500 V. The measured parameters for specific ion pairs are listed in Table S3 .

Statistical Analysis

All data are expressed as mean ± SEM. Statistical analyses were performed GraphPad Prism 8 (La Jolla, CA, USA). Significant differences of multiple groups were analyzed using one-way ANOVA followed by Dunnett post-hoc test or Kruskal–Wallis test followed by Dunn's post-hoc test for multiple comparisons. $P<0.05$ were considered statistically significant.

Results

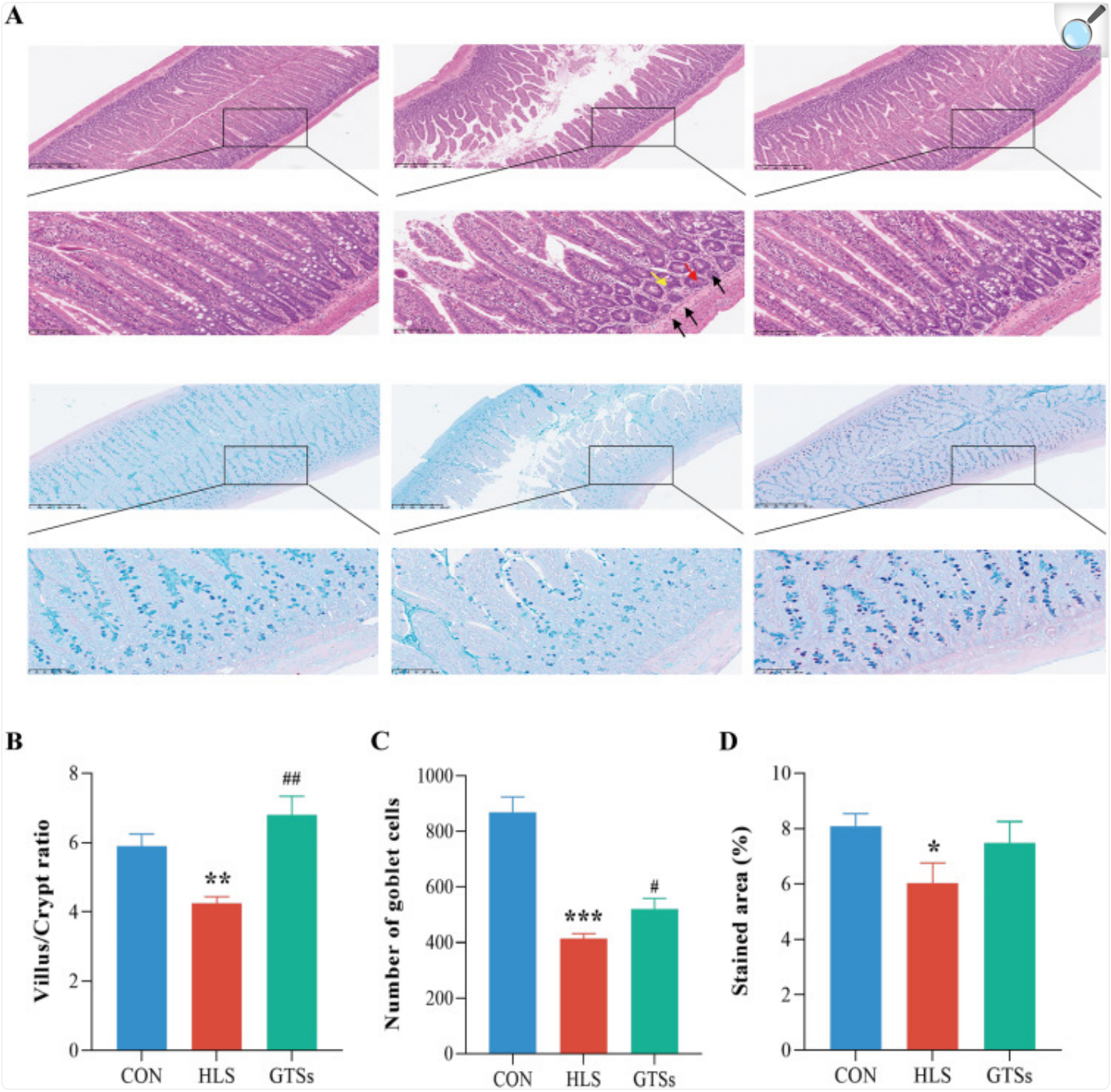
The Content of Eight Ginsenosides in GTSs Was Detected by UPLC-MS

The MRM chromatograms of ginsenosides Rb1, Rb2, Rg1, Rg2, Rc, Rd, Re, and Rf from GTSs were optimized for quantitative analysis. The calibration curve of the eight compounds showed good linearity with the available standards ($R^2 > 0.99$), and the lower limits of quantification for the ginsenosides ranged from 2.4414 to 312.5 ng/mL, indicating sufficient sensitivity for quantitative evaluation. As a result, It contained 258.89 mg/g Rb1, 65.77 mg/g Rb2, 12.87 mg/gRg1, 26.14 mg/g Rg2, 93.72 mg/g Rc, 90.90 mg/g Rd, 105.34 mg/g Re and 20.87 mg/g Rf (Figure S1).

Effect of GTSs on Intestinal Injury of Simulated Weightlessness Rats

As shown in Figure 1A, pathological analysis was performed to observe tissue damage. Compared to the CON group, obvious pathological damage occurred in the small intestine tissue of the HLS group, including typical small bowel villous atrophy, crypt inflammation, crypt dropout and crypt atrophy. However, GTSs effectively improved the villi/crypt ratio (Figure 1B) and alleviated the impaired intestinal symptoms.Mucin secreted by goblet cells is the first line of innate host defence. To confirm intestinal injury, we performed AB/PAS staining to evaluate the number of goblet cells (Figure 1A). In this study, significant goblet cell depletion and mucin reduction were observed in HLS group, which were reversed to varying degrees after GTSs treatment (Figure 1C and D).

Figure 1.



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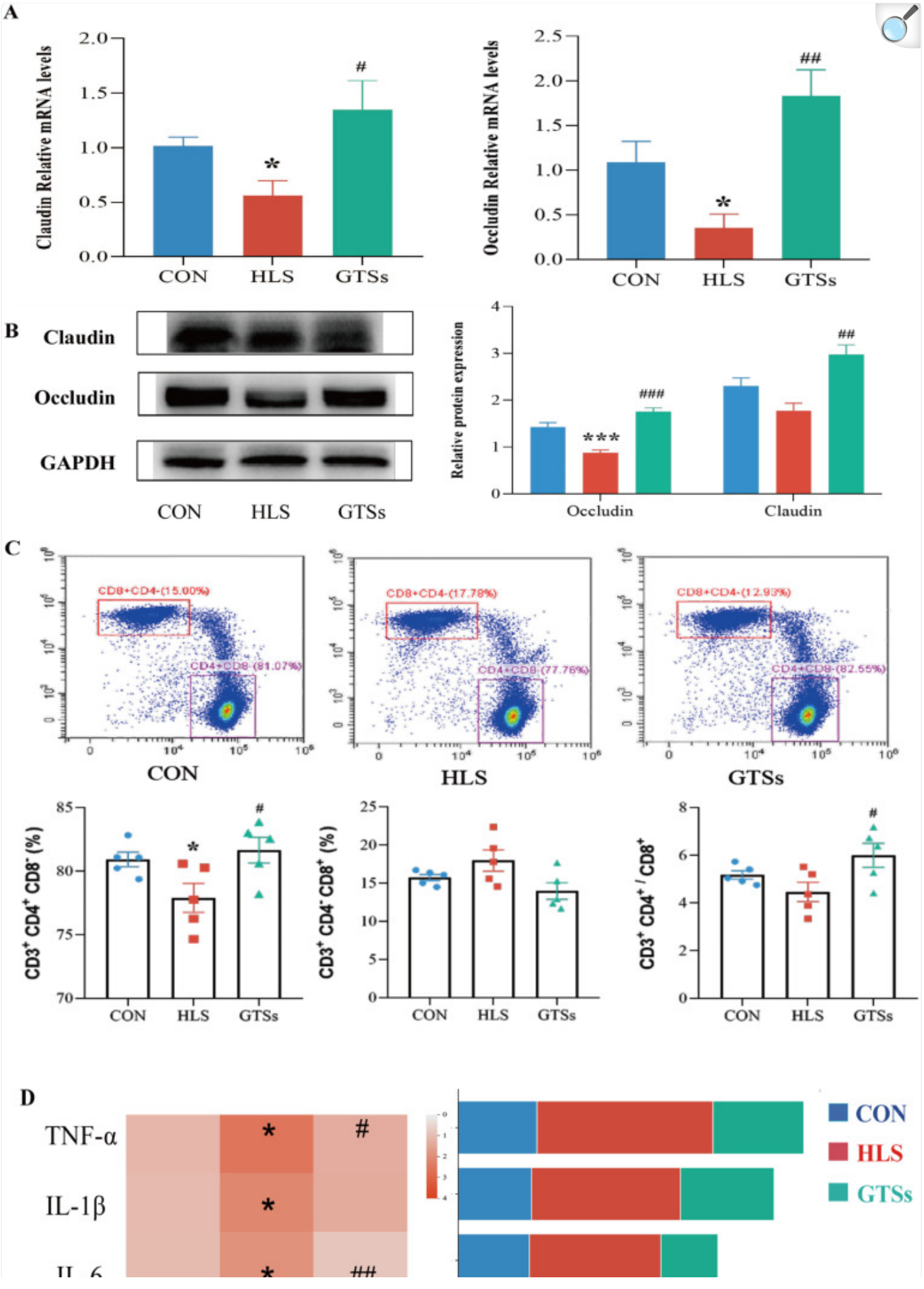
Ginsenosides can improve the histopathological changes of HLS rats. (**A**) Small intestine tissue for HE and AB-PSA staining (Top amplification: x5, Scale bar: 500 μm; Bottom amplification: x20, Scale bar: 100 μm). In HE staining, typical crypt inflammation (black arrow), crypt dropout (yellow arrow) and crypt atrophy (red arrow). In AB-PSA staining, goblet cells, responsible for secreting acidic mucin, are stained blue. (**B**) Villus/

crypt ratio of HE staining.(**C**) Numbers of goblet cells of AB-PAS staining. (**D**) AB-PAS staining area of acidic mucin. In each analysis, 6 random fields were selected for statistics. Quantitative data were expressed as the mean ± SEM from 6 rats in each group. *P < 0.05, **P < 0.01, ***P < 0.001, compared with CON group; $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, compared with HLS group.

Similarly, occludin and claudin, which are typical markers of tight junctions, were regulated by GTSs, were significantly increased after GTSs treatment (Figure 2A and B). The proportion of $CD3^{+}CD4^{+}$ T cells was significantly lower in the HLS group than that in the CON group, whereas the proportion of $CD3^{+}CD8^{+}$ T cells tended to increase (Figure 2C). GTSs regulated these changes close to normal levels, indicating that GTSs effectively restored the intestinal immune imbalance.

Figure 2.


A
Claudin Relative mRNA levels
2.0
1.5
1.0
0.5
0.0
#
*
CON
HLS
GTSs
Occludin Relative mRNA levels
2.5
2.0
1.5
1.0
0.5
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*
CON
HLS
GTSs
B
Claudin
Occludin
GAPDH
CON
HLS
GTSs
Relative protein expression
4
3
2
1
0
##
###
***
Occludin
Claudin
C
CD8+CD4-(15.00%)
CD4+CD8-(81.07%)
CON
CD8+CD4-(17.78%)
CD4+CD8-(77.76%)
HLS
CD8+CD4-(12.93%)
CD4+CD8-(82.55%)
GTSs
CD3+ CD4+ CD8- (%)
85
80
75
70
*
#
CON
HLS
GTSs
CD3+ CD4- CD8+ (%)
25
20
15
10
5
0
CON
HLS
GTSs
CD3+ CD4+ / CD8+
8
6
4
2
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TNF-α
IL-1β
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HLS
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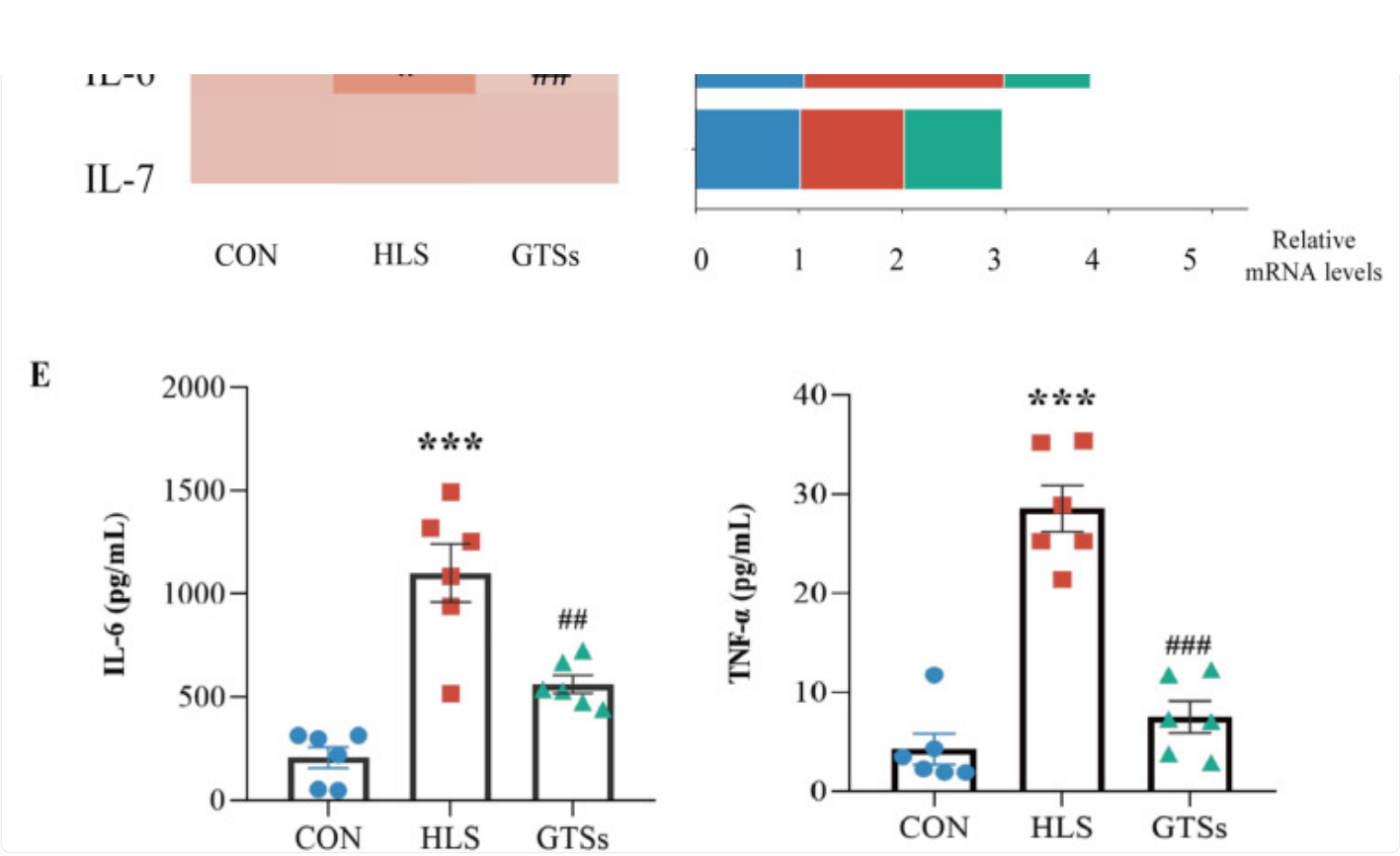

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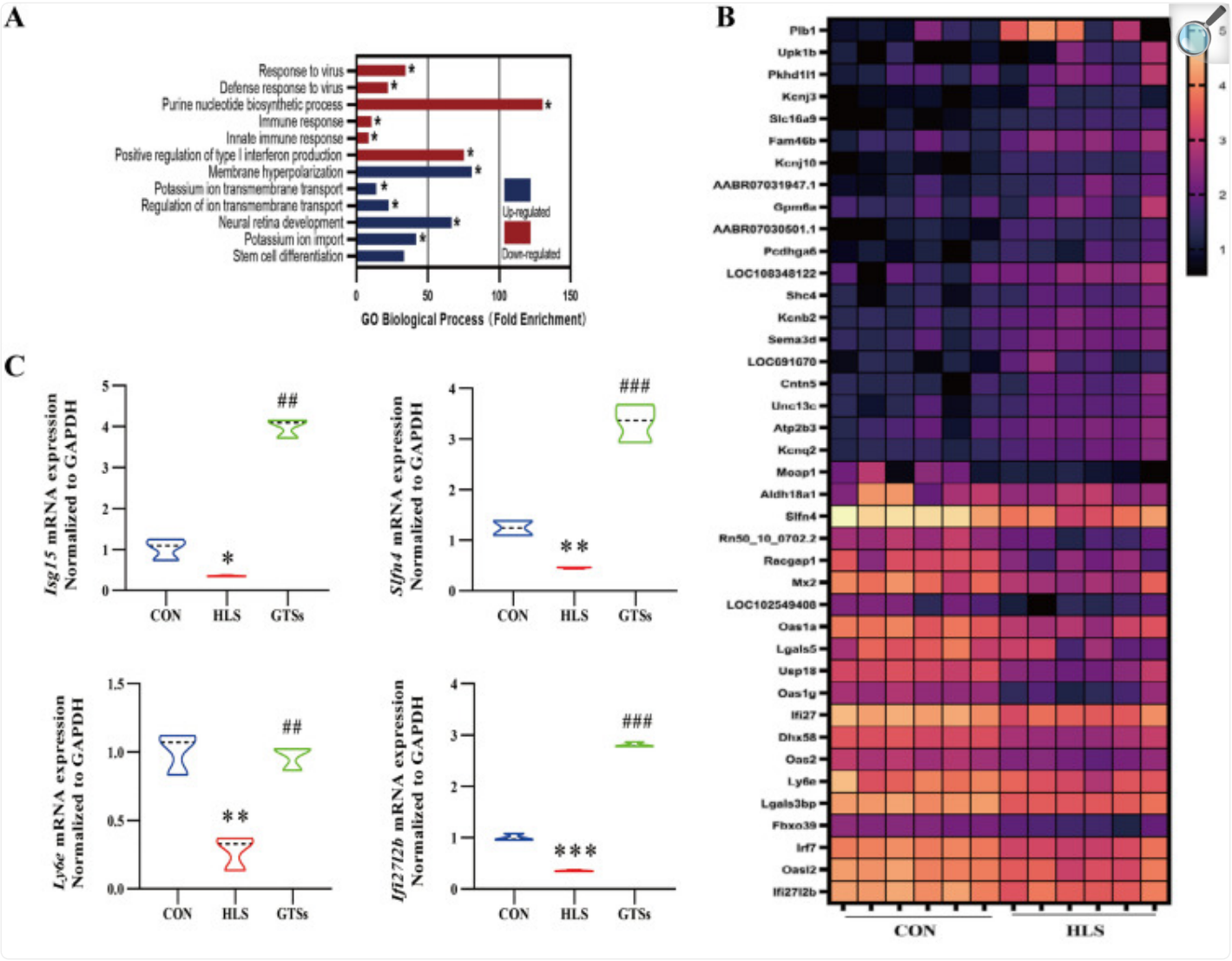
Ginsenosides effectively restored the intestinal injury in rats with simulated weightlessness. (**A**) The mRNA expression levels of the claudin and occludin genes in the small intestine. (**B**) Representative Western blotting images of occludin and claudin-1, and the relative protein expressions were normalized to GAP. (**C**) Presentative flow cytometry and proportions of CD3+, CD4+, and CD8+ cells in mesenteric lymph nodes. (**D**) The mRNA expression levels of four cytokine in the small intestine. A combined plot was used to display the results. They have the same vertical axis corresponding to different cytokines. While the horizontal axis represents experimental groups in the heatmap and the relative mRNA levels in the stacked bar chart. (**E**) The expressional levels of TNF-α and IL-6 in small intestine were determined by ELISA.Data are presented as the mean ± SEM, n=5–6. *P < 0.05, ***P < 0.001, compared with CON group; $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001, compared with HLS group.

Cytokines are capable of mediating intestinal immunity and epithelial crosstalk. RT-PCR was performed to determine the levels of TNF-α, IL-1β, IL-6, and IL-7. The contents of TNF-α and IL-6 in small intestine were analyzed by ELISA kit. As shown in Figure 2D–E, GTSs treatment reduced the expression of TNF-α and IL-6 in the HLS group. In conclusion, GTSs had a positive protective effect in rats with simulated weightlessness.

Effects of Simulated Weightlessness and GTSs on Gut Function

The small intestines of six rats were performed transcriptome in CON and HLS groups. Compared with the CON group, 660 genes were upregulated and 70 genes were downregulated in the HLS group. GO function enrichment analysis was performed to understand the biological processes of DEGs (Figure 3A). The results revealed that the downregulated genes were primarily associated with innate immune response. Upregulated genes are often found during the ion transmembrane transport. The top 40 differentially expressed genes were identified. The results showed that The levels of genes related to innate immunity (including Slfn4, Ly6e, Ifi2712, etc.) were significantly lower in the HLS group than in the CON group (Figure 3B). We performed RT-PCR analysis to validate the sequencing results and evaluate the therapeutic effects of GTSs. As shown in Figure 3C, HLS significantly decreased the expression of *Isg15, Slfn4, Ly6e* and *Ifi27l2b*, whereas the reduction in HLS rats were gradually reverted to normal levels after GTSs treatment.

Figure 3.



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The effect of GTSs on the gene expression in the small intestine of rats. (**A**) Biological processes of differentially expressed genes between the control and HLS groups based on GO enrichment analysis. (**B**) The heatmap of the top 40 differentially expressed genes. (**C**) RT-PCR analysis of genes associated with innate immunity. Data are presented as the mean ± SEM, n=6. *P < 0.05, **P < 0.01, ***P < 0.001, compared with CON group; $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001, compared with HLS group.

Structure Modulation of GTSs on Gut Microbial Under Simulated Weightlessness

We differentiated the data of each sample according to the barcode sequence, and the extracted data were saved in FASTQ format. The quality control data with reliable base calling are listed in Table S4 . The rarefaction curve indicated that the amount of sequencing data obtained in this study was reasonable (Figure S2). Alpha diversity, based on Shannon's analysis, was used to characterize the species diversity of the microbial communities. Strikingly, a sequential decrease in microbiota composition was observed in the HLS and GTSs groups (Figure 4A). However, there were differences in the relative abundance of dominant flora among the experimental groups, especially at the phylum and genus levels. At the phylum level, *Bacteroidetes* and *Firmicutes* dominated the fecal samples of all groups, and GTSs may have restored the relative abundance of these two species in rats subjected to weightlessness. Similarly, GTSs had modulatory effects on the dominant genera in the three groups, including *Lactobacillus, Tumorcoccus, Oscillospira and All0baculum* (Figure 4B). Furthermore, the Anosim test showed significant intergroup differences in bacterial community structure, and NMDS analysis was used to assess this change (Figure 4C). To further identify the most significant differential microbiota between the experimental groups, corresponding LEfSe analysis was performed (Figure 4D). The results showed that *Prevotella, Bifidobacterium, Staphylococcus, Corynebacterium* and *Jeotgalicoccus* were the dominant bacteria in the model group, and their relative abundance was significantly reduced after GTSs intervention (Table S5). In addition, compared with the CON group, *Lactobacillus, Lactobacillaceae and Bacilli* were identified as the major contributing flora in the GTSs group. These results suggest that GTSs have a dual modulatory effect on the gut microbial composition and abundance in simulated weightless rats. KEGG function prediction revealed that GTSs may be involved in signaling molecules and interactions, energy metabolism, immune function, and the nervous system by affecting the intestinal flora (Figure 4E).

Figure 4.

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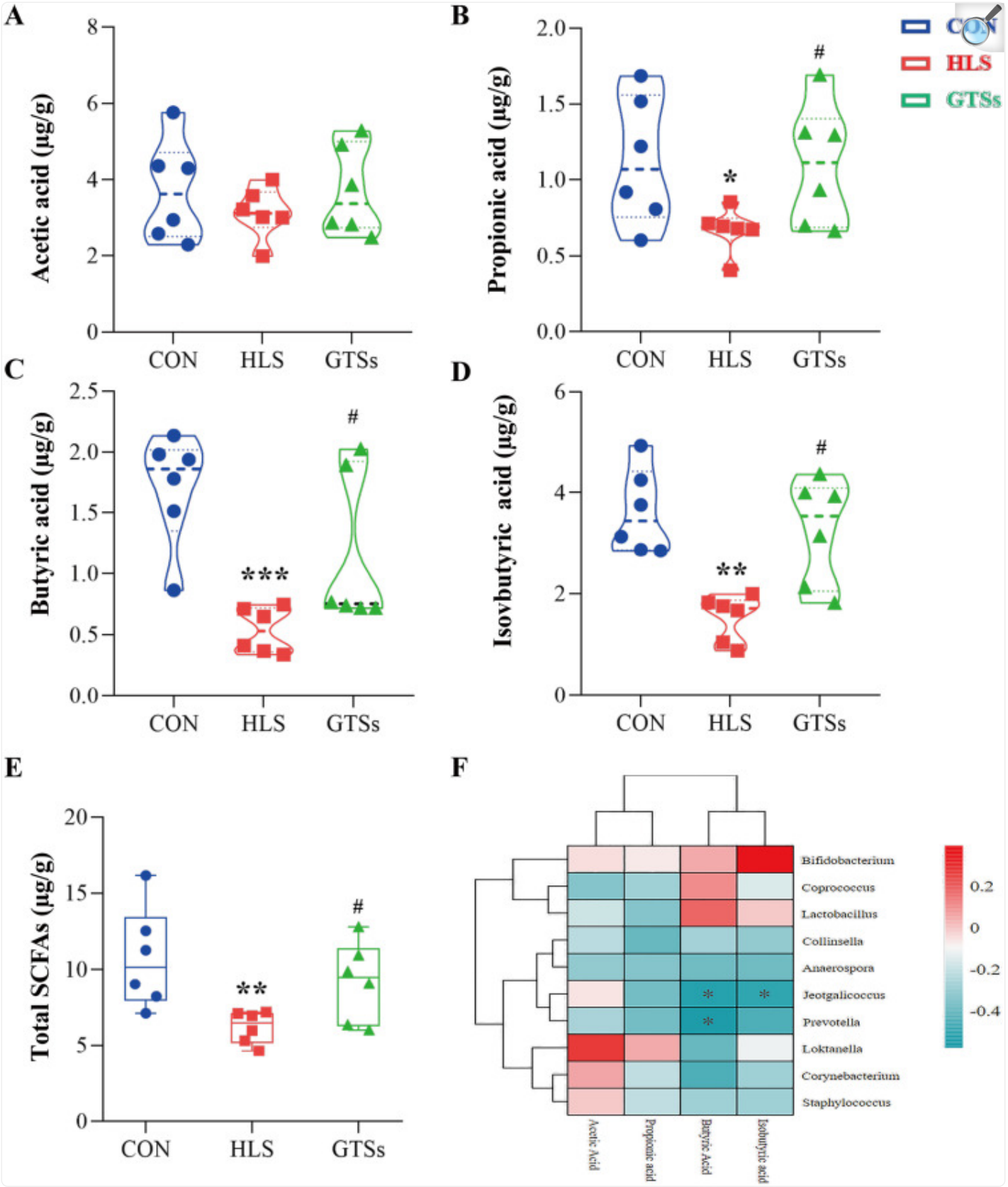
Effect of GTSs on gut microbiota diversity. (**A**) Alpha diversity analysis of the gut microbiota. (**B**) The species structure distribution at the phylum and genus levels. (**C**) β-Diversity analysis based on PCoA plot. (**D**) The cladogram generated from LEfSe analysis. (**E**) Distribution heatmap of microbiota. Six samples from each group were analyzed.

GTSs Improves Short-Chain Fatty Acid Content in Simulated Weightless Rats

Short-chain fatty acids (SCFAs) and microbial metabolites have been proven to have beneficial effects on the intestinal barrier and inflammation.[21] A reliable UPLC-ESI-MS/MS method was established to measure the contents of four major SCFAs (acetic acid, propionic acid, butyric acid, and isobutyric acid), which cumulatively accounted for 95% of the total SCFAs. The related figure and supporting data are presented in Figure S3 and Table S6, respectively. In addition to acetic acid, the contents of the other three SCFAs were significantly reduced in the HLS group, and GTS demonstrated the potential to restore SCFAs towards normal levels (Figure 5A–E). Correlation analysis was used to determine the contribution of the gut microbiota and $SCFA_S$ to the treatment of GTSs. In previous analyses, we found that GTSs not only dramatically regulated the relative abundance of the dominant flora in the HLS group but also had a unique regulatory flora. Figure 5F shows a strong negative correlation between butyric acid and gut microbiota, and the correlation coefficients of *Prevotella* and *Jeotgalicoccus* were more significant.

Figure 5.


A
Acetic acid (μg/g)
CON
HLS
GTSs
B
Propionic acid (μg/g)
C
Butyric acid (μg/g)
D
Isovbutyric acid (μg/g)
E
Total SCFAs (μg/g)
F
Bifidobacterium
Coprococcus
Lactobacillus
Collinsella
Anaerospora
Jeotgalicoccus
Prevotella
Loktanella
Corynebacterium
Staphylococcus
Acetic Acid
Propionic acid
Butyric Acid
Isobutyric acid

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GTSs affect the levels of SCFAs and the association with gut microbiota. (**A**) Acetic acid. (**B**) Propionic acid. (**C**) Butyric acid. (**D**) Isobutyric acid. (**E**) Total acid. (**F**) Correlation analysis of SCFAs and intestinal flora. Data are presented as the mean ± SEM, n=5. *P < 0.05, **P < 0.01, ***P < 0.001, compared with CON group; #P < 0.05, compared with HLS group.

Discussion

Long-term microgravity exposure causes adverse effects on the immune system. The innate immune system, which is composed of granulocytes, natural killer cells, monocytes, macrophages and dendritic cells, is the first line of defense for the body to initiate self-protection.22 It can not only recognize invading pathogens, but also sense and interact with the environment.23 Previous reports have suggested that more than 50% of astronauts on the first manned missions showed increased susceptibility to microbial infections caused by impaired innate immune systems.24 In addition, recent studies have further demonstrated that microgravity can impair the differentiation, function and signal transduction of innate immune cells.23 For example, the analysis of peripheral blood from astronauts showed that space flight would lead to impaired cell migration and antigen presentation mediated by monocytes in innate immunity.22 The intestinal innate immune system encompasses barrier defenses serviced by the epithelial cell layer, as well as bactericidal immune cell populations, including macrophages and neutrophils.25 Breakdowns in intestinal homeostasis driven by weightlessness are related to immune system perturbations.7 Intestinal immune cells are distributed in the intestinal epithelium, lamina propria, and lymphoid tissue.26 Mesenteric lymph nodes (MLD), which are located in lymphoid tissues, are sites of intestinal adaptive immune responses induced by intestinal microbiomes and mediate inflammatory responses.27 $CD4^+$ and $CD8^+$ T cells are also important contributors. In this study, GTSs significantly increased the proportion of $CD3^+CD4^+$ T cells in MLD and improved the $CD4^+$ /$CD8^+$ ratio, suggesting that GTSs could enhance the cell-mediated immune response of MLD and the intestinal immune barrier. In addition, transcriptomic analysis identified innate immune pathways significantly associated with HLS and confirmed the downregulation of immune-related genes (*Ly6e, Isg15* and *Ifi27l2b*) in the HLS group. To validate this outcome, we performed RT-PCR analysis and obtained similar results. Interestingly, GTSs effectively reversed the expression of these genes, suggesting its therapeutic potential. *Ly6e* exhibits transcriptional activity in numerous tissues, including the liver and intestine.28 Importantly, the biological functions of *Ly6e* have been linked to immunoregulatory functions, particularly T-cell proliferation and activation. *Ly6e*-loaded DCS effectively stimulated T Cell subsets and significantly increased the proportion of Th1 cells, which play an important role in colorectal cancer with immune cell infiltration.29 Interferon alpha inducible protein 27 (*IFI27*), including *Ifi2712b*, is responsible for a variety of biological processes including innate immunity and apoptosis.30,31 *Liu* et al investigated the protective effects of the poria cocos-derived polysaccharide, CMP33, against IBD and identified multiple regulatory genes including Ifi27l2b.32 *Slfn4* belongs to the Schlafen (SLFNs) family, a family of proteins strongly induced by type 1 interferons (IFN-α) and has been implicated in

myeloid cell differentiation, proliferation, and immune responses.[33,34] Although much attention has been paid to the antiviral properties of *Isg15*, recent reports have also suggested that uncoupled *Isg15* may act as a signalling molecule to stimulate immunomodulatory responses, such as neutrophil chemotaxis and activation of inflammatory cytokines.[35] Our results showed that the expression of these genes significantly increased after GTSs treatment, suggesting that GTSs can protect intestinal immunity in a weightless environment.

The intestinal microecology is co-defined with the intestinal barrier, intestinal microorganisms, and intestinal metabolites.[36] Previous studies described the effects of weightlessness on these factors. Jin et al indicated that impaired intestinal mucosal barrier function caused by weightlessness is closely related to the intestinal villi and tight junction structures.[7] In several tail-suspension rat models, researchers have observed typical pathologic features, such as intestinal villus shedding, decreased number of goblet cells, and inflammatory cell infiltration.[7,37] At the same time, the expression levels of tight junction proteins were significantly decreased and inflammatory cytokines were increased. Similar changes were observed in the gene expression levels of tight junction proteins (claudin and occludin) and pro-inflammatory factors (TNF-α, IL-1β, and IL-6). HE staining confirmed the pathological injury of the small intestine in the HLS group, as well as the recovery potential of GTSs. Moreover, deficiency in mucin synthesis and secretion by goblet cells directly leads to intestinal mucosal barrier dysfunction. GTSs effectively restored the reduction in goblet cells caused by weightlessness, which is another factor that supports the protective function of the intestinal barrier.

At the gut microbes, a reduction in microbial diversity and beneficial species of Akkermansia and Lactobacillus was observed.[4] Similar results were obtained in this study. For both endemic flora present only in the HLS group (*Bifidobacterium, Staphylococcus, g-prevotella, Corynebacterium, Jeotgalicoccus*) and flora with variations in abundance (*Lactobacillus, Ruminococcus*), GTSs showed strong positive regulatory effects. *Staphylococcus* is an opportunistic pathogen that enhances intestinal permeability of intestine.[38] Similarly, *Prevotella* colonization exacerbates intestinal inflammation and systemic autoimmune diseases.[39] *Lactobacillus* has been identified as a versatile probiotic that participates in improving intestinal barrier function, immune regulation, metabolic regulation, and other biological processes.[40] Clinically, *Lactobacillus* has been used to ameliorate intestinal disorders such as diarrhea, inflammatory bowel disease, and irritable bowel syndrome.[41–43] Recent studies have shown that changes in the abundance of *lactobacilli, ruminococcus*, and *Oscillospira* directly affect intestinal permeability, systemic inflammation, and macrophage dysfunction.[44,45] Notably, GTSs treatment reduced the abundance of these potentially pathogenic bacteria while increasing the content of beneficial bacteria, which is beneficial for maintaining gut microbial homeostasis and protecting host health. In addition, we found that GTSs independently regulated *Lactobacillaceae* and *Bacilli*, which have been shown to be beneficial for regulating intestinal immunity or inflammation.[46,47] Taken together, these results indicate that GTSs can effectively restore the intestinal flora disorders induced by simulated microgravity. KEGG function prediction also confirmed that these bacteria are involved in immune processes.

Short-chain fatty acids (SCFAs), including acetic acid, propionic acid, and butyric acid, are mediators of communication between intestinal signals and immune functions, accounting for more than 90% of the total intestinal SCFAs.[48] Acetic

acid not only provides an important energy source for host cells but also protects intestinal epithelial cells by increasing the expression of tight junction proteins, thereby reducing intestinal permeability.[49] Propionic acid can regulate intestinal inflammation, tight junction proteins, and mucin production to maintain the intestinal barrier function.[50–52] Butyric acid has been shown to have a potent anti-inflammatory effect, which is closely related to intestinal permeability.[53] In the present study, weightlessness caused a significant reduction in the levels of these SCFAs, which were antagonized by GTSs. A strong correlation was observed between butyric acid-*Jeotgalicoccus* and butyric acid-*Prevotella*. As a harmful bacterium,[54] GTSs intervention reduced the abundance of *Jeotgalicoccus* in vivo, while the butyric acid content increased at the metabolic level, which confirmed the rationality of their negative correlation. *Prevotella* is directly related to butyric acid synthesis in vivo.[55] Notably, our results showed inconsistent trends between *Prevotella* and butyric acid, which might be related to the regulatory effects of GTS_S on other butyric acid-producing bacteria. These results further highlight the potential role of GTSs in maintaining intestinal barrier integrity. Based on this result, we will conduct more detailed experiments to investigate the specific regulatory pathways of GTSs on intestinal immunity, gut microbiota and microbiota metabolites. It will help to investigate the effects of natural ingredients from food on the health and homeostasis of astronauts, and to play a better role in the operated spaceflight business.

Conclusion

GTSs can improve intestinal microecological disorders and impair immune function caused by weightlessness. The underlying mechanism may be related to the “intestinal immune microbiota-metabolic” pathway. However, a more profound mechanism needs to be explored.

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Abbreviations

GTSs, Ginseng total saponins; HLS, hind limb suspension; MLD, Mesenteric lymph nodes; SCFAs, Short-chain fatty acids.

Data Sharing Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas, took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. Yanli Wang, Tian Chen, and Zhe Shi are the co-first authors, and Qin Hu and Ying Chen are the corresponding authors.

Disclosure

The authors declare that there are no conflicts of interest in this work.

References

1. Zhang Q, Ding L, Sun C. et al. Exit the frog, enter the human: physiology and experimental psychology in nineteenth-century astronomy. *Br J Hist Sci*. 2001;201:445–453. [Google Scholar]

2. Li Y, Liu S, Deng Y, Deng Y, Deng Y. Dragon's Blood Regulates Rac1-WAVE2-Arp2/3 Signaling Pathway to Protect Rat Intestinal Epithelial Barrier Dysfunction Induced by Simulated Microgravity. *Int J mol Sci*. 2021;22(5):2722. doi: 10.3390/ijms22052722 [DOI] [PMC free article] [PubMed] [Google Scholar]

3. Alvarez R, Stork CA, Sayoc-Becerra A, Marchelletta RR, Prisk GK, McCole DF. A Simulated Microgravity Environment Causes a Sustained Defect in Epithelial Barrier Function. *Sci Rep*. 2019;9(1):17531. doi: 10.1038/s41598-019-53862-3 [DOI] [PMC free article] [PubMed] [Google Scholar]

4. Shama S, Qaisar R, Khan NA, Tauseef I, Siddiqui R. The Role of 4-Phenylbutyric Acid in Gut Microbial Dysbiosis in a Mouse Model of Simulated Microgravity. *Life*. 2022;12:1301. [DOI] [PMC free article] [PubMed] [Google Scholar]

5. Ramos-Nascimento A, Grenga L, Haange SB, et al. Human gut microbiome and metabolite dynamics under simulated microgravity. *Gut Microbes*. 2023;15(2):2259033. doi: 10.1080/19490976.2023.2259033 [DOI] [PMC free article] [PubMed] [Google Scholar]

6. Yuan L, Zhang R, Li X, et al. Long-term simulated microgravity alters gut microbiota and metabolome in mice. *Front Microbiol*. 2023;14:1100747. doi: 10.3389/fmicb.2023.1100747 [DOI] [PMC free article]

[PubMed] [Google Scholar]

7. Jin M, Zhang H, Zhao K, et al. Responses of Intestinal Mucosal Barrier Functions of Rats to Simulated Weightlessness. *Front Physiol*. 2018;9:729. doi: 10.3389/fphys.2018.00729 [DOI] [PMC free article] [PubMed] [Google Scholar]

8. Wang J, Han C, Lu Z, et al. Simulated microgravity suppresses MAPK pathway-mediated innate immune response to bacterial infection and induces gut microbiota dysbiosis. *FASEB J*. 2020;34(11):14631–14644. doi: 10.1096/fj.202001428R [DOI] [PubMed] [Google Scholar]

9. Jin M, Wang J, Zhao K, Zhou H, Zhao K. Simulated Weightlessness Perturbs the Intestinal Metabolomic Profile of Rats. *Front Physiol*. 2019;10:1279. doi: 10.3389/fphys.2019.01279 [DOI] [PMC free article] [PubMed] [Google Scholar]

10. Che Q, Luo T, Shi J, He Y, Xu DL. Mechanisms by Which Traditional Chinese Medicines Influence the Intestinal Flora and Intestinal Barrier. *Front Cell Infect Microbiol*. 2022;12:863779. doi: 10.3389/fcimb.2022.863779 [DOI] [PMC free article] [PubMed] [Google Scholar]

11. Wang F, Roh YS. Mitochondrial connection to ginsenosides. *Arch Pharm Res*. 2020;43(10):1031–1045. doi: 10.1007/s12272-020-01279-2 [DOI] [PubMed] [Google Scholar]

12. Ratan ZA, Haidere MF, Hong YH. Pharmacological potential of ginseng and its major component ginsenosides. *J Ginseng Res*. 2021;45(2):199–210. doi: 10.1016/j.jgr.2020.02.004 [DOI] [PMC free article] [PubMed] [Google Scholar]

13. Zhao L, Zhang T, Zhang K. Pharmacological effects of ginseng and ginsenosides on intestinal inflammation and the immune system. *Front Immunol*. 2024;15:1353614. doi: 10.3389/fimmu.2024.1353614 [DOI] [PMC free article] [PubMed] [Google Scholar]

14. Zhao L, Sui M, Zhang T, Zhang K. The interaction between ginseng and gut microbiota. *Front Nutr*. 2023;10:1301468. doi: 10.3389/fnut.2023.1301468 [DOI] [PMC free article] [PubMed] [Google Scholar]

15. Cheng H, Liu J, Zhang D, et al. Ginsenoside Rg1 Alleviates Acute Ulcerative Colitis by Modulating Gut Microbiota and Microbial Tryptophan Metabolism. *Front Immunol*. 2022;13:817600. doi: 10.3389/fimmu.2022.817600 [DOI] [PMC free article] [PubMed] [Google Scholar]

16. Bai X, Fu R, Duan Z, Wang P, Zhu C, Fan D. Ginsenoside Rk3 alleviates gut microbiota dysbiosis and colonic inflammation in antibiotic-treated mice. *Food Res Int*. 2021;146:110465. doi: 10.1016/j.foodres.2021.110465 [DOI] [PubMed] [Google Scholar]

17. Zhang L, Li F, Qin WJ, Fu C, Zhang XL. Changes in intestinal microbiota affect metabolism of

ginsenoside Re. *Biomed Chromatogr*. 2018;32(10):e4284. doi: 10.1002/bmc.4284 [DOI] [PubMed] [Google Scholar]

18. Dong LJC, Chen Y, Hu Q, et al. Protective effects of ginsenosides on macrophages subjected to simulated weightlessness. *Digital Chin Med*. 2023;6:416–425. [Google Scholar]

19. Feng L, Liu XM, Cao FR, et al. Anti-stress effects of ginseng total saponins on hindlimb-unloaded rats assessed by a metabolomics study. *J Ethnopharmacol*. 2016;188:39–47. doi: 10.1016/j.jep.2016.04.028 [DOI] [PubMed] [Google Scholar]

20. Jiang N, Lv J, Zhang Y, Sun X, Yao C, Liu X. Protective effects of ginsenosides Rg1 and Rb1 against cognitive impairment induced by simulated microgravity in rats. *Front Pharmacol*. 2023;14:1167398. doi: 10.3389/fphar.2023.1167398 [DOI] [PMC free article] [PubMed] [Google Scholar]

21. Pearce SC, Karl JP, Weber GJ. Effects of short-chain fatty acids on intestinal function in an enteroid model of hypoxia. *Front Physiol*. 2022;13:1056233. doi: 10.3389/fphys.2022.1056233 [DOI] [PMC free article] [PubMed] [Google Scholar]

22. ElGindi M, Sapudom J, Ibrahim IH, et al. May the Force Be with You (Or Not): the Immune System under Microgravity. *Cells*. 2021;10(8):1941. doi: 10.3390/cells10081941 [DOI] [PMC free article] [PubMed] [Google Scholar]

23. Duscher AA, Vroom MM, Foster JS. Impact of modeled microgravity stress on innate immunity in a beneficial animal-microbe symbiosis. *Sci Rep*. 2024;14(1):2912. doi: 10.1038/s41598-024-53477-3 [DOI] [PMC free article] [PubMed] [Google Scholar]

24. Paulsen K, Thiel C, Timm J, et al. Microgravity-induced alterations in signal transduction in cells of the immune system. *Acta Astronaut*. 2010;67(9–10):1116–1125. doi: 10.1016/j.actaastro.2010.06.053 [DOI] [Google Scholar]

25. Roh TT, Chen Y, Rudolph S, Gee M, Kaplan DL. InVitro Models of Intestine Innate Immunity. *Trends Biotechnol*. 2021;39(3):274–285. doi: 10.1016/j.tibtech.2020.07.009 [DOI] [PMC free article] [PubMed] [Google Scholar]

26. Wang Q, Lu Q, Jia S, Zhao M. Gut immune microenvironment and autoimmunity. *Int Immunopharmacol*. 2023;124:110842. doi: 10.1016/j.intimp.2023.110842 [DOI] [PubMed] [Google Scholar]

27. Cai G, Wusiman A, Gu P, et al. Supplementation of Alhagi honey polysaccharides contributes to the improvement of the intestinal immunity regulating the structure of intestinal flora in mice. *Food Funct*. 2021;12(20):9693–9707. doi: 10.1039/D1FO01860D [DOI] [PubMed] [Google Scholar]

28. Li T, Liu W, Wang C, et al. Multidimension Analysis of the Prognostic Value, Immune Regulatory Function, and ceRNA Network of LY6E in Individuals with Colorectal Cancer. *J Immunol Res*. 2022;2022:5164265. doi: 10.1155/2022/5164265 [DOI] [PMC free article] [PubMed] [Google Scholar]

29. Tokhanbigli S, Asadirad A, Baghaei K, et al. Dendritic Cell-Based Therapy Using LY6E Peptide with a Putative Role Against Colorectal Cancer. *Immunotargets Ther*. 2020;9:95–104. doi: 10.2147/ITT.S245913 [DOI] [PMC free article] [PubMed] [Google Scholar]

30. Huang S, OuYang H, Ma Z, OuYang H, Ma Z, OuYang H. Interferon alpha-inducible protein 27 (IFI27) is a prognostic marker for pancreatic cancer based on comprehensive bioinformatics analysis. *Bioengineered*. 2021;12(1):8515–8528. doi: 10.1080/21655979.2021.1985858 [DOI] [PMC free article] [PubMed] [Google Scholar]

31. Gytz H, Hansen MF, Skovbjerg S, et al. Apoptotic properties of the type 1 interferon induced family of human mitochondrial membrane ISG12 proteins. *Biol Cell*. 2017;109(2):94–112. doi: 10.1111/boc.201600034 [DOI] [PubMed] [Google Scholar]

32. Liu X, Yu X, Xu X, Zhang X, Zhang X. The protective effects of Poria cocos-derived polysaccharide CMP33 against IBD in mice and its molecular mechanism. *Food Funct*. 2018;9(11):5936–5949. doi: 10.1039/C8FO01604F [DOI] [PubMed] [Google Scholar]

33. Xiang X, Wu Y, Li H, Li C, Yan L, Li Q. Plasmacytoid Dendritic Cell-Derived Type I Interferon Is Involved in Helicobacter pylori Infection-Induced Differentiation of Schlafen 4-Expressing Myeloid-Derived Suppressor Cells. *Infect Immun*. 2021;89(11):e0040721. doi: 10.1128/IAI.00407-21 [DOI] [PMC free article] [PubMed] [Google Scholar]

34. Ding L, Sheriff S, Sontz RA, Merchant JL. Schlafen4(+)-MDSC in Helicobacter-induced gastric metaplasia reveals role for GTPases. *Front Immunol*. 2023;14:1139391. doi: 10.3389/fimmu.2023.1139391 [DOI] [PMC free article] [PubMed] [Google Scholar]

35. Wardlaw CP, Petrini JHJ. ISG15: a link between innate immune signaling, DNA replication, and genome stability. *Bioessays*. 2023;45(7):e2300042. doi: 10.1002/bies.202300042 [DOI] [PMC free article] [PubMed] [Google Scholar]

36. Dang K, Zhang N, Gao H, Wang G, Liang H, Xue M. Influence of intestinal microecology in the development of gout or hyperuricemia and the potential therapeutic targets. *Int J Rheum Dis*. 2023;26(10):1911–1922. doi: 10.1111/1756-185X.14888 [DOI] [PubMed] [Google Scholar]

37. Wang S, Wang R, Li GQ, Cho JL, Deng Y, Li Y. Myosin light chain kinase mediates intestinal barrier dysfunction following simulated microgravity based on proteomic strategy. *J Proteomics*. 2021;231:104001. doi: 10.1016/j.jprot.2020.104001 [DOI] [PubMed] [Google Scholar]

38. Li Z, Dong J, Wang M, Yan J, Li H. Resveratrol ameliorates liver fibrosis induced by nonpathogenic Staphylococcus in BALB/c mice through inhibiting its growth. *Mol Med*. 2022;28:52. doi: 10.1186/s10020-022-00463-y [DOI] [PMC free article] [PubMed] [Google Scholar]

39. Iljazovic A, Roy U, Galvez EJC, et al. Perturbation of the gut microbiome by Prevotella spp. enhances host susceptibility to mucosal inflammation. *Mucosal Immunol*. 2021;14(1):113–124. doi: 10.1038/s41385-020-0296-4 [DOI] [PMC free article] [PubMed] [Google Scholar]

40. Huang R, Wu F, Zhou Q, et al. Lactobacillus and intestinal diseases: mechanisms of action and clinical applications. *Microbiol Res*. 2022;260:127019. doi: 10.1016/j.micres.2022.127019 [DOI] [PubMed] [Google Scholar]

41. Gutierrez-Castrellon P, Lopez-Velazquez G, Diaz-Garcia L, Jimenez-Gutierrez C, Parra M. Diarrhea in Preschool Children and Lactobacillus reuteri: a Randomized Controlled Trial. *Pediatrics*. 2014;133(4):e904–909. doi: 10.1542/peds.2013-0652 [DOI] [PubMed] [Google Scholar]

42. Zocco MA, Dal Verme LZ, Cremonini F, et al. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther*. 2006;23(11):1567–1574. doi: 10.1111/j.1365-2036.2006.02927.x [DOI] [PubMed] [Google Scholar]

43. Martoni CJ, Srivastava S, Leyer GJ. Lactobacillus acidophilus DDS-1 and Bifidobacterium lactis UABla-12 Improve Abdominal Pain Severity and Symptomology in Irritable Bowel Syndrome: randomized Controlled Trial. *Nutrients*. 2020;12(2):363. doi: 10.3390/nu12020363 [DOI] [PMC free article] [PubMed] [Google Scholar]

44. Thevaranjan N, Puchta A, Schulz C, et al. Age-Associated Microbial Dysbiosis Promotes Intestinal Permeability, Systemic Inflammation, and Macrophage Dysfunction. *Cell Host Microbe*. 2018;23(4):570. doi: 10.1016/j.chom.2018.03.006 [DOI] [PMC free article] [PubMed] [Google Scholar]

45. Santisteban MM, Qi Y, Zubcevic J, et al. Raizada, Hypertension-Linked Pathophysiological Alterations in the Gut. *Circ Res*. 2017;120(2):312–323. doi: 10.1161/CIRCRESAHA.116.309006 [DOI] [PMC free article] [PubMed] [Google Scholar]

46. Yu B, Zhang D, Wu Y, et al. A new polysaccharide from Hawk tea: structural characterization and immunomodulatory activity associated with regulating gut microbiota. *Food Chem*. 2023;418:135917. doi: 10.1016/j.foodchem.2023.135917 [DOI] [PubMed] [Google Scholar]

47. Huang W, Deng Z, Lu L, et al. Polysaccharides from soybean residue fermented by Neurospora crassa alleviate DSS-induced gut barrier damage and microbiota disturbance in mice. *Food Funct*. 2022;13(10):5739–5751. doi: 10.1039/D2FO00137C [DOI] [PubMed] [Google Scholar]

48. Parada Venegas D, Landskron G, Gonzalez MJ, et al. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front Immunol*. 2019;10:277. doi: 10.3389/fimmu.2019.00277 [DOI] [PMC free article] [PubMed] [Google Scholar]

49. Laffin M, Fedorak R, Zalasky A, Park H, Hotte N, Madsen KL. A high-sugar diet rapidly enhances susceptibility to colitis via depletion of luminal short-chain fatty acids in mice. *Sci Rep*. 2019;9(1):12294. doi: 10.1038/s41598-019-48749-2 [DOI] [PMC free article] [PubMed] [Google Scholar]

50. Vinolo MA, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC, Curi R. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. *J Nutr Biochem*. 2011;22(9):849–855. doi: 10.1016/j.jnutbio.2010.07.009 [DOI] [PubMed] [Google Scholar]

51. Ma X, Fan PX, Li LS, Qiao SY, Zhang GL, Li DF. Butyrate promotes the recovering of intestinal wound healing through its positive effect on the tight junctions. *J Anim Sci*. 2012;90(4):266–268. doi: 10.2527/jas.50965 [DOI] [PubMed] [Google Scholar]

52. Willemsen LE, Koetsier MA, van Deventer SJ, van Tol EA. Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. *Gut*. 2003;52(10):1442–1447. doi: 10.1136/gut.52.10.1442 [DOI] [PMC free article] [PubMed] [Google Scholar]

53. Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr*. 2009;139(9):1619–1625. doi: 10.3945/jn.109.104638 [DOI] [PMC free article] [PubMed] [Google Scholar]

54. Zhao S, Peng X, Zhou QY, et al. Bacillus coagulans 13002 and fructo-oligosaccharides improve the immunity of mice with immunosuppression induced by cyclophosphamide through modulating intestinal-derived and fecal microbiota. *Food Res Int*. 2021;140:109793. doi: 10.1016/j.foodres.2020.109793 [DOI] [PubMed] [Google Scholar]

55. Huang CC, Shen MH, Chen SK, et al. Gut butyrate-producing organisms correlate to Placenta Specific 8 protein: importance to colorectal cancer progression. *J Adv Res*. 2020;22:7–20. doi: 10.1016/j.jare.2019.11.005 [DOI] [PMC free article] [PubMed] [Google Scholar]

Associated Data

This section collects any data citations, data availability statements, or supplementary materials included in this article.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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